IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 023533/0113

In re patent application of

CORNETT *et al.*

Serial No. 09/783,580

Filed: July 21, 2000

Group Art Unit: 1648

Examiner: Scott David Priebe

For: RECOMBINANT BETA2-ADRENERGIC RECEPTOR DELIVERY AND USE IN TREATING AIRWAY AND VASCULAR DISEASES

DECLARATION UNDER 37 CFR § 1.132

Commissioner for Patents
PO Box 1450
Alexandria, Virginia 22313-1450

I, Lawrence E. Cornett, hereby declare:

1. I am an inventor of the captioned application. I have worked in the field of adrenergic receptor structure and function, including how adrenergic receptor gene expression is regulated in mammalian tissues that are responsive to circulating catecholamines since 1977. I have published over 32 papers in this field. Attached as Exhibit A is my curriculum vitae.

2. I have read and understood the Final Rejection dated January 15, 2003 and particularly the Examiner's comments on page 5-7, regarding the lack of evidence of the expression of β_2 adrenergic receptor (β_2AR) *in vivo*, and the Advisory Action dated May 22, 2003, in the above-identified application. In support of the expression of β_2AR *in vivo*, I provide the following experimental data.

3. Twenty, adult Sprague Dawley rats at stable weights of ~400g were divided into 5 groups (n=4 per group) based on treatment: Group 1 - saline treatment via endotracheal bolus; Group 2 - AAV2 treatment via endotracheal bolus; Group 3 - AAV2 treatment via endotracheal nebulization; Group 4 - AAV1 treatment via endotracheal bolus; and Group 5 - AAV1 treatment via endotracheal nebulization. AAV1 and AAV2 represent two different serotypes of AAV and, currently there are six known AAV serotypes. Nebulization was performed in-line with the ventilator using the Aeroneb Lab device (provided by Aerogen, Inc.). A total of 240 µl of vector

containing the human -β_2AR gene (AAV-β_2AR/EGFP) or saline was delivered to the airway in 80 µl aliquots followed by ventilation for 5 minutes and rotation of the animal between each delivery to maximize distribution. Eighteen animals completed the study. One saline control animal expired after baseline measurement due to tracheal puncture, and one AAV1 animal was eliminated due to anesthesia issues at follow-up. All other animals were recovered after the first data collection and completed second data collection. Mean body weight was 421g at baseline and was 455g 4 weeks after treatment, representing a 9% change in body weight over the 4 week study period.

Respiratory function was measured, using the FlexiVent System (SCIREO, Montreal, Quebec, Canada) before, and at 4 weeks after gene delivery for changes in lung function, including change in airway dynamic resistance (representing total airway resistance) and for change in Newtonian resistance (a measure of central airway resistance). Because it minimizes ventilator dead space, the FlexiVent System is a superior method to obtaining flow measurements in small animals compared to the more traditional methods using pneumotachographs. After 4 weeks, all treatment groups showed a modest fall in dynamic airway resistance (data not shown), but with no statistically significant differences between treatment groups. The modest drop in airway dynamic resistance likely reflects the modest change in airway size with slight increase in weight and airway diameter. The central airway resistance measurements in AAV-β_2AR treated rats and control-rats are presented in Figure 1 (Exhibit B). Both the AAV2 nebulization group (Group 3) and AAV1 nebulization group (Group 5) demonstrate a fall in Newtonian resistance in 3 of 4 rats in Group 3 (See Figure 1, *Panel B*) and in 3 of 3 rats in Group 5. (See Figure 1, *Panel C*). In the 3 control animals, there was no consistent change in Newtonian resistance over the 4 week period of the study (See Figure 1, *Panel A*). Animals treated with AAV1 or AAV2 via bolus showed no significant change in resistance (data not shown).

4. These conclusions are supported by the results obtained with the immunohistochemistry experiments shown in Figure 2 (Exhibit C). To determine efficiency of target protein expression, lungs were harvested, and immunohistochemistry was performed by standard techniques using a monoclonal antibody to enhanced green fluorescent protein (EGFP) and a polyclonal antibody specific to the human β_2AR cytoplasmic terminus. The secondary antibody was anti-mouse-

horseradish peroxidase antisera to detect the EGFP and anti-rabbit AF594 to detect the human β_2AR. The data shows a distribution of target protein expression of both EGFP and human β_2AR between groups, 4 weeks after treatment with AAV1 using nebulization (Figure 2, upper panels) and AAV2 using nebulization (Figure 2, center panels) of both EGFP and human β_2AR expression as compared to saline treated animals (Figure 2, bottom panels). Saline treated animals showed no EGFP or human β_2AR expression. In addition, preliminary review of histologic samples is devoid of evidence of an inflammatory response in all animals 4 weeks after AAV delivery to the airway.

5, These data presented in Figures 1 and 2 show that human β_2AR is expressed in the cells of rat lungs *in vivo*. It is my opinion that these data confirm that the expression of human β_2AR in the lungs is responsible for decreased central airway resistance, which is evidence of a therapeutic effect in rat lungs.

6. I hereby declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

By: L. E. Cornett

Date: 10/16/03

Lawrence E. Cornett, Ph.D.